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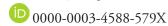
Salinity Tolerance of Different Silage Hybrids Maize Cultivars

Silajlık Hibrit Mısır Çeşitlerinin Tuzluluğa Toleransı

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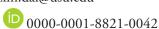
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ABSTRACT

Since corn is sensitive to salt and saline irrigation water, soil salinity poses a serious threat to corn production. Therefore, it is important to know in advance the response of the cultivated corn varieties to salt stress in order not to lose too much in production. In this study, it was aimed to determine the response to salt (NaCl) stress of thirteen commercial silage maize hybrid varieties commonly used by farmers in Türkiye. For this purpose, seed germination and early seedling growth were tested by exposing the plants to different salt stress levels (0, 40, 80 and 120 mM) for 14 days. Three b asic groups (Biomass, S eedling G rowth Vigor and Seed Germination) were established by factor analysis (FA) for the nine parameters -seed germination percentage (sgp), mean germination time (mgt), shoot lenght (sl), root lenght (rl), fresh weight (fw), dry weight(dw), dry matter (dm), plant water content (pwc), vigor index (vi)- tested for seed germination and seedling growth. As a result of the two-way analysis of variance with heterogeneous variance on standardized factor scores; it was determined that 'Dekalb-7211' hybrid cultivar showed the best performance and "Dekalb-7240" hybrid cultivar showed the worst performance in different salt stress levels tested in terms of three main groups formed. The results of this research will contribute to the determination of stress-tolerant varieties, which is one of the important and first steps of maize breeding.

Keywords: Maize, salinity, factor analysis (FA), two-way ANOVA, heterogeneous variance



ÖZET

Mısır tuza ve tuzlu sulama suyuna hassas bir bitki olduğundan, toprak tuzluluğu mısır üretimi için önemli bir tehlike oluşturmaktadır. Dolayısıyla, üretimde çok fazla verim kaybına uğramamak için ekimi yapılan mısır çeşitlerinin tuz stresine karşı tepkisinin önceden bilinmesi önem arz etmektedir. Bu çalışmada, Türkiye'de çiftçiler tarafından yaygın olarak kullanılan on üç ticari silajlık mısır hibrit çeşidinin tuz (NaCl) stresine tepkisinin belirlenmesi amaçlanmıştır. Bu amaçla bitkiler 14 gün boyunca farklı tuz stres seviyelerine (0, 40, 80 ve 120 mM) maruz bırakılarak tohum çimlenmesi ve erken dönem fide gelişimleri test edilmiştir. Tohum çimlenmesi ve fide gelişiminde test edilen dokuz parametreleri -tohum çimlenme yüzdesi (tçy), ortalama çimlenme süresi (oçs), sürgün uzunluğu (su), kök uzunluğu (ku), taze ağırlık (ta), kuru ağırlık (ka), kuru madde (km), bitki su içeriği (bsi), canlılık indeksi (vi)için faktör analizi (FA) ile üç temel grup (Biyokütle, Fide Büyüme Gücü ve Tohum Çimlenmesi) oluşturulmuştur. Standartlaştırılmış faktör puanları üzerinde heterojen çeşit varyansı ile yapılan iki yönlü varyans analizi sonucunda; oluşturulan üç temel grup bakımından test edilen farklı tuz stresi seviyelerinde en iyi performansı 'Dekalb-7211' hibrit çeşidinin, en kötü performansı ise "Dekalb-7240" hibrit çeşidinin gösterdiği tespit edilmiştir. Bu araştırma sonuçları, mısır ıslahının önemli ve ilk basamaklarından biri olan stres faktörüne karşı toleranslı çeşitlerin belirlenmesine katkı sağlayacak niteliktedir.

Anahtar kelimeler: Mısır, tuzluluk, faktör analizi, ikiyönlü ANOVA, heterojen varyansı

INTRODUCTION

Salinity affects roughly 800 million hectares of soil worldwide according to the survey by the Food and Agriculture Organization of the United Nation (Hernández, 2019) and restricts crop growth and yield (Hütsch et al., 2014). Maize (*Zea mays* L.) is classed as moderately susceptible to salinity, as it belongs to the C4 metabolism group of plants (Turk and Alagöz 2020). It is the third most important cereal crop in the world, providing basic food to millions of people and half of all energy use.

Furthermore, it is an important raw material in the textile, paper, and feed industries (Al Samsul Huqe et al., 2021). Salt stress causes significant yield loss in maize each year (Luo et al., 2017). Therefore, it is important to understand the response of maize to salt stress with morphological, physiological, biochemical and molecular studies. The study of salt tolerance in significant maize hybrids used in agricultural production is an important first stage in maize breeding (Luo et al., 2017). For many plants, including maize, tolerance to salt stress during germination and early seedling stage are the most critical periods (Farooq et al., 2015; Luo et al., 2017). To screen salt tolerant maize hybrids under salt stress, genetic variability for germination under salt stress and characteristics such as morpoholgical parameters should be used (Giaveno et al., 2007). Multivariate analyzes such as principal component analysis (PCA) and cluster analysis have been widely used to screen for salt tolerance of different plant genotypes (Aslam et al., 2017; Kaya et al., 2019; Güngör et al., 2021; Bres et al., 2022) because using multiple parameters improves accuracy and provides a general tolerance level based on mean values generated from each stress level (Kaya et al., 2019). Chaum and Kirdmanee (2010) and Luo et al., (2017) showed that multivariate cluster analysis can be used to classify the salt tolerance of maize hybrids during seed germination and morphological parameters. The aim of this study was to screen different silage maize hybrids by evaluating their responses in germination and early seedling growth stage to increasing salt stress. This study will contribute to the screening and/or identification of tolerant varieties, a first, critical step in maize breeding studies.

MATERIALS AND METHODS

Plant Materials

In this study, 13 commercial silage hybrids maize cultivars obtained from the Kırşehir Ahi Evran University, Faculty of Agriculture, Department of Field Crops (Türkiye) were used as plant material. Silage hybrids maize cultivars used in the research and some of their traits are shown in Table 1.



Table 1. Silage hybrids maize cultivars used in the research and FAO maturity group of these cultivars

Cultivars	Cultivars of source	FAO maturity group of cultivar				
Kilowatt	KWS seeds	It is a cultivar that can be grown as silage and grain in the FAO-700 death group (100-110 days).				
LG-30500	Limagrain	It is a cultivar in the FAO-550 group (120-130 days).				
Doge	KWS seeds	It is a cultivar in the FAO-700+ group.				
PL-524	Polen Seeds	It is a cultivar in the FAO-500-550 group.				
Dekalb-7211	DEKALB	It is a cultivar in the FAO-750 group.				
Dekalb-7240	DEKALB	It is a cultivar in the FAO-750 group.				
Samada-07	Republic of Türkiye Ministry of Agriculture and Forestry Black Sea Agricultural Research Institute	It is a cultivar in the FAO-600 group.				
PL-472	Polen Seeds	It is a cultivar in the FAO-500-550 group.				
Kerbanis	KWS seeds	It is a cultivar in the FAO-450 group.				
9628 HP F1	Biotek seeds	It is a cultivar in the FAO-500 group.				
Klips	KWS seeds	It is a cultivar in the FAO-670-700 group.				
Macha-DMR108	Polen Seeds	It is a cultivar in the FAO-480 group.				
Adasa-16	Republic of Türkiye Ministry of Agriculture And Forestry Eastern Mediterranean Agricultural Research Institute	It is a cultivar in the FAO 700 group.				

Methods

The study was carried out in a completely randomized design with 3 replications per combined cultivar and salt treatment. Five seeds of each genotype were put onto three layers of filter paper. Each filter paper packet was irrigated with 10 mL of one aqueous saline solution (0, 40, 80, and 120 mM NaCl). To prevent evaporation, each filter paper packet of seeds was rolled and placed in a sealed plastic bag. To prevent salt buildup after incubation, each rolled paper was replaced every 2 days. Before planting, the seeds were treated with fungicide (Thiram 80% WP). Germination experiments were carried out in the dark at 26±1 °C in incubator (Memmert-In110). When the sprouting radicle lengthened to 2 mm, the seed was said to have germinated. For 14 days, germination % was tracked every 24 hours (ISTA, 2003). To measure the rate of germination, mean germination time (MGT) was computed in accordance with Ellis and Roberts (1980). MGT= $\Sigma(Dn)/\Sigma n$, where n is the number of seeds that just started to sprout on day D, and D is the number of days since the start. In 14-day-old seedlings, seedling shoot and root length and seedling fresh and dry weights were measured. Dry weights were calculated after samples were dried in an oven at 70°C for 48 hours (Beyaz et al., 2011). Plant water content (pwc), dry matter ratio (%) (dw) and vigor index (vi) were calculated according to the following formulas, respectively:

Plant water content (pwc) = (Fresh weight–Dry weight)/ Fresh weight×100 (Zheng et al., 2008)

Dry matter (dm) ratio= (Dry weight/Fresh weight)*100 (Bres et al., 2022)

Vigor index (vi) = (average root lenght +average hypocotyl length) x germination percentage (gp) (Abdul-Baki and Anderson 1973)

Statistical Analysis

The nine responses (sgp, mgt, sl, rl, fw, dw, dm, pwc and v1) were analyzed with PROC FACTOR in SAS/STAT 15.1 (SAS Institute Inc., Cary, NC) to group the responses into



a reduced number of underlying constructs (Table 2.). The factor analysis was performed on standardized responses. Factors were extracted with the principle component method coupled with varimax rotation. Three factors were formed as shown in Table 1 and characterized as follows: Factor 1 is a biomass construct loaded primarily from seedling length (in cm), root length (cm), fresh weight (g) and vigour index; Factor 2 reveals seedling growth-strength by the contrast between dry weight (g) and dry matter (%) loadings with plant water content (%) loading; Factor 3

indicates seed germination by the constrast between seed germination (%) loading with mean germination time (day) loading. The three factors explain 82.4% of the total variability in the nine responses. Factor scores for each observation were computed with PROC SCORE in SAS/STAT. Since the responses were standardized, zero score of each factor indicates the average level of that factor, with a positive score above average and a negative score below average.

Table 2. Rotated factor pattern with loadings from the nine responses. Loading in bold show defining responses for each factor.

Responses	Factor1 (Biomass -size)	Factor2 (Seedling growth-strength)	Factor3 (Seed germination)		
Seed germination percentage (%)	0.120	-0.294	0.741		
Mean germination time (day)	0.047	-0.154	-0.713		
Shoot legth (cm)	0.857	-0.305	-0.067		
Root length (cm)	0.860	-0.299	0.081		
Fresh weight (mg per plant)	0.952	0.030	-0.084		
Dry weight (mg per plant)	0.268	0.932	-0.108		
Dry matter percentage (%)	-0.552	0.745	0.073		
Plant water content (%)	0.536	-0.802	-0.017		
Vigour index	0.832	0.229	0.392		

The factor scores were analyzed by two-way-ANOVA with heterogeous error variance by cultivar using PROC GLIMMIX in SAS/STAT to examine the differences among treatment groups. Simple effects of cultivar and salt concentration were compared pairwise with Tukey-Kramer's method to adjust for multiplicity. Significant level is specified at p<0.05 level.

RESULTS AND DISCUSSION

Table 3. summarized the results to test salt concentration impacts on each hybrid maize cultivars for each of the three factors: Factor 1 (biomass), Factor 2 (seedling strength) and Factor 3 (seed germination). For each factor, estimated factor scores with the same lowercase letter within each a cultivar implied that the cultivar growth was not infuenced by salt stress. Cultivar comparisons under each salt concentration for each factor are illustrated in Figure 1. Vertical reference lines at score zero represent the average level of each factor. Cultivars with positive scores implied above average performance and negative below average performance. Cultivars sharing the same upper case letter

within each plot did not statistically difference from each other

Biomass Score (Factor1)

Increased salinity did not affect the biomass score (Factor 1) of hybrids cvs. 9628 HP F1, Dekalb-7211, Dekalb-7240, Klips, Samada-07, and Macha-DMR108 (Table 3.). On the other hand, Doge, Kerbanis, Kilowatt, LG-30500, PL-472, PL-524 hybrid maize cultivars were significantly (p<0.05) affected by increasing salt concentrations (Table 3.). Under the highest salt concentration at 120 mM, biomass score for these cultivars were significantly lower than those under control conditions. Although increasing salt concentration



generally decreased maize biomass score, PL-524 improved its biomass with salt and had the best biomass performance at the 40 mM salt level. Similarly, Adasa-16 had the best biomass under 40 mM salt treatment with estimated score of 1.01, significantly higher than its score at 120 mM salt stress of 0.02. It's also notable that Klips, although its biomass score not was impacted by salt levels, had the poorest biomass score around -2 persistently.

The four plots in the first row of Figure 1 showed biomass comparisons of the thirteen maize cultivars under each of the four salt concentrations. Klips is consistently low in the biomass scores regardless of salt levels and significantly different from other cultivars. Klips, therefore, is a weak cultivar and not recommended. When no salt stress (0 mM) was applied, Kilowatt and Samada-07 had leading biomass scores. As the salt concentrations increased, however, biomass scores dropped for Kilowatt and were below avarage at the 80 and 120 mM salt concentrations. Samada-07, however, kept the leading biomass score among the cultivars under all levels of salt stress. PL-524 also outperformed other cultivars by improving biomass score from below average under control conditions to leading under salt stress levels. The tolerance of PL-524 to increased salinity may be due to its genetic makeup.

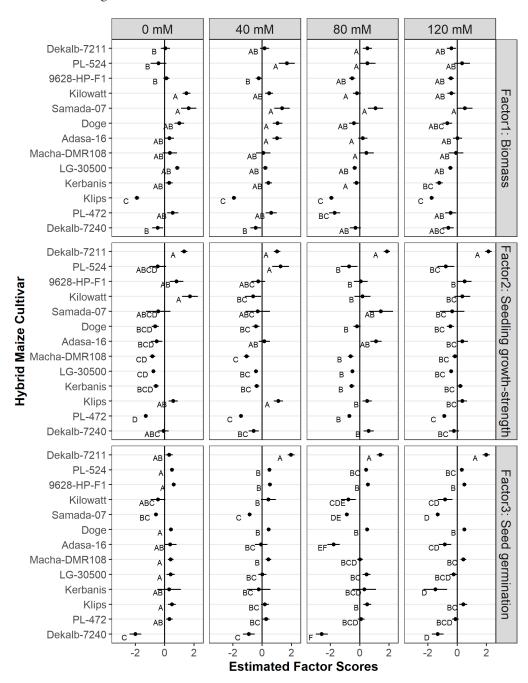




Figure 1. Comparison of the thirteen silage hybrids maize cultivars under each saline (NaCl) treatments at 0 (control), 40, 80 and 120 mM for the three factors: biomass (Factor 1), seedling growth-strength (Factor 2), and seed germination (Factor 3). Plots are arranged with saline concentrations as columns, and the three factors as rows. X-axis is the estimated standardized factor scores. The

verticle reference line at zero represents the average level of each factor. Y-axis is the hybrid maize cultivars. The lines across the dots represent standard error bars. Within each plot, estimated factor scores with the same uppercase letter are not statistically different at p<0.05 level by pairwise comparison with Tukey-Kramer's method for multiplicty adjustment.

Table 3. Estimated standardized biomass score (Factor 1), seedling strength score (Factor 2) and seed germination score (Factor 3) of the thirteen silage hybrids maize cultivars under four saline (NaCl) treatments at 0 (control), 40, 80, and 120 mM.

	Factor1: Biomass					Factor2: Seedling growth- strength			Factor3: Seed germination			
Cultivars	0 mM	40 mM	80 mM	120 mM	0 mM	40 mM	80 mM	120 mM	0 mM	40 mM	80 mM	120 mM
9628 HP F1	0.11 ^{a*}	-0.23ª	-0.51ª	-0.43ª	0.81ª	-0.27ª	0.08ª	0.50ª	0.62ª	0.52ab	0.56ab	0.48 ^b
Dekalb-7211	0.04ª	0.16ª	0.53ª	-0.39ª	1.33 ^{bc}	1.01°	1.85 ^{ab}	2.13ª	0.30 ^b	1.97ª	1.40ª	1.98ª
Dekalb-7240	-0.48ª	-0.44ª	-0.29ª	-0.61ª	-0.08ab	-0.58 ^b	0.61ª	-0.22 ab	-2.00ab	-0.91ª	-2.58 ^b	-1.33 ^{ab}
Doge	1.00ª	1.04ª	-0.39b	-0.67 ^b	-0.64ª	-0.42ª	-0.18ª	-0.46ª	0.43 ^b	$0.44^{\rm b}$	0.51ª	0.49ab
Kerbanis	0.31ª	0.43ª	-0.21ª	-1.23 ^b	-0.59b	-0.37 ^b	-0.54 ^b	0.21ª	0.30ª	-0.23ª	0.31ª	-1.49ª
Kilowatt	1.49ª	0.47 ^b	-0.19 ^b	-0.40 ^b	1.72ª	-0.62 ^b	0.19 ^{ab}	0.35 ^{ab}	-0.45ª	0.43ª	-0.77ª	-0.83ª
Klips	-1.89ª	-1.92ª	-1.93ª	-1.74ª	0.58ª	1.10ª	0.51ª	0.35ª	0.49ª	0.19ª	0.51ª	0.41ª
LG-30500	0.86ª	0.21 ^b	-0.34°	-0.46°	-0.76 ^b	-0.42ª	-0.49ª	-0.42ª	0.41ª	0.01ª	0.47ª	-0.24ª
PL-472	0.55ª	0.61ª	-1.70 ^b	-0.45 ^b	-1.28 ^{bc}	-1.45°	-0.70ª	-0.88 ^{ab}	0.32ª	0.26ª	0.10ª	-0.12ª
PL-524	-0.42 ^b	1.68ª	0.53 ^{ab}	0.33 ^{ab}	-0.47 ^b	1.25ª	-0.72 ^b	-0.77 ^b	0.50ª	0.49ª	0.43ª	0.31ª
Samada-07	1.64ª	1.35ª	1.08ª	0.52ª	-0.44ª	-0.30ª	1.44ª	-0.35ª	-0.60ª	-0.85ª	-0.87ª	-1.32 ^b
Adasa-16	0.33^{ab}	1.01ª	0.21ab	0.02 ^b	-0.54 ^b	0.15 ^{ab}	1.12ª	0.35 ^{ab}	0.38ª	-0.07 ^{ab}	-1.78°	-0.84 ^{bc}
Macha-DMR108	0.35ª	0.07ª	0.45ª	-0.09ª	-0.83 ^b	-1.06 ^b	-0.62ab	-0.16ª	0.41ª	0.42ª	0.02ª	0.41ª

^{*}For each factor, estimated standardized scores with the same lowercase letter within a cultivar are not statistically different at p<0.05 level by pairwise comparison with Tukey-Kramer's method for multiplicty adjustment.

Similarly, Dekalb-7211 while not a biomass leading cultivar at 0 mM salt treatment, increased its biomass scores when salt level increased and became the leading cultivar under the 80 mM salt treatment. Changes in plant biomass during the early seedling development period is an important point in terms of the plant's response to stress factors and therefore its tolerance (Farooq et al., 2015).

In term of estimated standardized biomass score (Factor 1), the response of the investigated hybrid maize cultivars to salinity stress were different. However, the results of this study showed that increased salinity had a negative impact on biomass in many hybrid maize cultivars. Adverse effect of salinity on biomass of maize genotypes has been reported previously (Giaveno et al., 2007; Cha-um and Kirdmanee 2010; Luo et al., 2017; Turk and Alagöz 2020).



Seedling growth- strength score (Factor 2)

Elevating salinity did not impact seedling growth-strength for 9628 HP F1, Doge, and Klips and Samada-07 hybrid cultivars but increasing salt concentration resulted in improved seedling growth-strength for Dekalb-7211, Dekalb-7240, Kerbanis, PL-472, Samada-07, Adasa-16, and Macha-DMR108. Adverse impact of seedling growth-strength were observed in PL-524 and Kilowatt. The best seedling score is estimated to be 1.25 at 40 mM salt treatment for PL-524. The scores decreased to less than-0.70 at higher salt concentrations and the differences are statistically significant. Estimated seedling score is 1.72 for Kilowatt when no salt treatment was applied. The scores dropped significantly when salt treatment was added.

The second row of plots in Figure 1 shows the hybrid cultivar comparisons at each salt treatment level. Without salt stress, Dekalb-7211, Kilowatt and 9628 HP F1 are the three top cultivars with estimated seedling scores close to or above 1.0. As salt stress was applied and became more intense, Dekalb-7211 continued to have high seedling scores and the scores are significantly higher than those for the rest of the cultivars.

Results for Factor 2 showed agreement with several researchers (Giaveno et al., 2007, Cha-um and Kirdmanee 2010, Luo et al., 2017, Turk and Alagöz 2020) who found that different cereal genotypes responded variably in their seedling growth-strength measurements against salinity levels. Although Kumar et al., (2008) found that seedling dry weights and plant water content, two contributing measures to Factor 2, are tolerent of salt stress regardless of genotype, our results showed several cultivars in this study are susceptible under salt treatment. The salt stress can either increase seedling growth-strength as found in several cultivars such as Dekalb-7211 or degrade seedling growth-strength as found in Kilowatt.

Seed germination score (Factor 3)

Salinity is one of the primary factors slowing down seed germination and reducing the overall germination rate (Rahman et al., 2000). Testing for salt tolerance at the earliest phases of plant growth is crucial because seeds

that germinate more quickly in salty environments may be anticipated to establish themselves more quickly, producing larger yields (Petrović et al. 2016). One of the main goals of this study was to identify different silage maize cultivars that were sensitive to salt and tolerant to salt during germination in order to assess their potential for salt tolerance. NaCl concentrations affected the germination of the examined silage hybrid maize cultivars (Table 3). These cultivars' sensitivities to salt content, however, varied. Estimated standardized seed germination scores (Factor 3) were not affected by salt stress in silage hybrid maize cultivars Kerbanis, Kilowatt, Klips, LG-30500, PI-472, PI-524 and Macha-DMR108 (Table 3). For the remaining cultivars, increased salt level adversely affected seed germination except for Dekalb-7211. Seed germination improved under the salt treatments with estimated scores for Dekalb-7211. The estimated scores ranged from 1.40 - 1.98, significantly higher than 0.30 under no salt condition. This is also shown in Figure 1 with the four plots in the bottom row. Although not a strong seed germinator under control treatment, Dekalb-7211 outperformed other cultivars and became the leading cultivar in seed germination under the investigated salt treatment concentrations 40, 80 and 120 mM. The plots revealed that Dekalb-7240 has the poorest seed germination property with or without salt stress.

The decrease in the water potential gradient between the seeds and their surrounding media is most likely what is causing the rising concentration of NaCl (Bradford, 1995; Cokkızgın, 2012). The higher osmotic potential prevented water absorption that was necessary for seed development (Güngör et al. 2021). Furthermore, salinity stress impairs seed germination either ionically through the buildup of Na⁺ and Cl⁻ or osmotically through reduced water absorption, which results in an imbalance in nutrient uptake and toxicity impact (Shokohifard et al. 1989; Cokkızgın, 2012). Additionally, alterations in enzyme activity might be brought on by salinity due to the toxicity of ions (Gomes-Filho et al. 2008; Dehnavi et al. 2021). The alteration of the metabolism of nucleic acids and proteins, the disruption of the hormonal balance, and other significant alterations in plant germination are all brought on by this disruption of enzyme processes (Dantas et al.



2007; Ryu and Cho 2015; Dehnavi et al. 2021). Generally, our results showed that salinity has negative effects on hybrid maize seed germination. However, the current study's findings were in line with those of other researchers, who found that cereal crops' cultivar germination rates declined as salt concentrations increased (Giaveno et al., 2007; Cha-um and Kirdmanee, 2010; Luo et al., 2017; Turk and Alagöz, 2020; Dehnavi et al. 2020). Interestingly, while some hybrid cultivars (Doge, 9628 HP F1, Klips, and Macha-DMR108) had above average performance in terms of seed germination at different salt levels, they underperformed for biomass (Factor 1) and seedling strength (Factor 2). According to Giaveno et al., (2007), germination has genetic diversity, however there is no link between germination and early seedling growth under salt stress. Our results are in parallel with the results of this study.

CONCLUSION

In this study, the responses of different silage hybrid maize cultivars (Kilowatt, LG-30500, Doge, PL-524, Dekalb-7211, Samada-07, PL-472, Kerbanis, Dekalb-7240, 9628 HP F1, Klips, Macha-DMR108, and Adasa-16) to four levels of salt stress (NaCl concentrations of 0, 40, 80, and 120 mM) were summarized as biomass, seedling growth-strength, and seed germination. Each cultivar's performance on the three aspects of growth and strength are independent and should all be considered when recommending salt tolerant genotypes.

Hybrid maize cultivar Dekalb-7211 grew better and stronger than other hybrid maize cultivars under salt stress and is thus recommended. Future study on this crop can focus on improving its biomass regardless of salt conditions. Dekalb-7240, on the other hand, is not recommended due to its low scores in biomass, seedling growth-strength and germination. Large variations were observed in some hybrid cultivars such as Samada-07 and Kerbanis, indicating that more samples are needed to estimate their salt tolerant performance in the future. A crop's response under salt stress is influenced by its physiological, biochemical and molecular characteristics. Governing mechanisms for Dekalb-7211 having the greatest tolerance to salinity stress are unknown and require further study. Also, salt stress studies under field conditions should be carried out in

order to test these cultivars against salinity in an applied growing environment. In addition, it is recommended that drought stress, the sister of salt stress, of these hybrid silage maize cultivars be tested. This study might be valuable contribution to the knowledge of maize breeding.

Conflict of Interest

The authors declared that there is no conflict of interest.

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